

IN THE CLAIMS

1. (original) A modified bacterial surface layer (S-layer) protein, the modification comprising the internal insertion of a heterologous polypeptide.
2. (original) The protein according to claim 1 wherein:
 - (a) the unmodified protein is from a Gram positive or non aquatic bacteria, optionally a lactic acid bacteria or *Lactobacillus*; and/or
 - (b) the heterologous polypeptide is a functional polypeptide or a polypeptide of interest, optionally a binding or targeting protein (such as an antigen, antibody, or part thereof).
3. (previously presented) A protein according to claim 1 wherein:
 - (c) the protein retains most of the full length sequence of the unmodified S-layer protein;
 - (d) the polypeptide is inserted at an internal location at least five amino acids from the C or N terminus; and/or
 - (e) the modified protein has a size of from 40 to 70 kDa.
4. (previously presented) A protein according to claim 1 which:
 - (f) has a crystallisation or C-terminal domain that is predominantly basic, or hydrophobic,
 - (g) an N-terminal domain which is either predominantly hydrophilic; or
 - (h) has alternating hydrophobic and hydrophilic regions.
5. (previously presented) A protein according to claim 1 wherein the heterologous polypeptide is inserted at a location in the protein either so that it is :
 - (i) exposed, or present on the cell surface;
 - (j) present in the surface layer, or the cell wall;

(k) is protected from external proteolytic processing or is not recognised or bound by external antibodies.

6. (previously presented) A protein according to claim 1 wherein the modified or unmodified protein:

- (l) crystallises, optionally into an oblique lattice (such as of p1 or p2 symmetry);
- (m) has a cell wall anchor domain;
- (n) has a pI of at least 7; and/or
- (o) is predominantly basic.

7. (previously presented) A protein according to claim 1 wherein the polypeptide comprises an antigen causing or specific for a disease, and optionally is an antigen recognisable by an antibody or is all or part of an antigen from an anaerobic bacteria, optionally *Clostridium*.

8. (previously presented) A protein according to claim 1 which (in unmodified form) is from *Lactobacillus acidophilus*, *crispatus*, *helveticus*, *amylovorus*, or *gallinarum*.

9. (currently amended) A fragment of a bacterial surface layer (S-layer) protein which is:

- a) an N-terminal fragment or a fragment that is capable of forming a dimer with another such fragment or a trimer with two other such fragments;
- b) capable of forming dimers with another such fragment and either
 - (i) includes an immunodominant or exposed loop region and is from 20 to 200 amino acids long; or
 - (ii) excludes an entire immunodominant ~~immunodominant~~ or exposed loop region and is from 20 to 155 amino acids long.

10. (previously presented) A polynucleotide encoding a protein according to claim 1.

11. (original) A vector comprising a polynucleotide according to claim 10.
12. (original) A host cell comprising, or which is has been transformed with a vector according to claim 11.
13. (currently amended) A bacteria expressing a surface layer protein (or fragment) according to claim 1 or a [[A]] fragment of a bacterial surface layer (S-layer) protein which is:
- a) an N-terminal fragment or a fragment that is capable of forming a dimer with an other such fragment or a trimer with two other such fragments;
 - b) capable of forming dimers with another such fragment and either
 - (i) includes an immunodominant or exposed loop region and is from 20 to 200 amino acids long; or
 - (ii) excludes an entire immunodominant ~~immunodominant~~ loop region and is from 20 to 155 amino acids long.
14. (original) A bacteria according to claim 13 which is a lactic acid bacteria, optionally from *Lactobacillus*, and is preferably *L. plantarum*, *L. acidophilus* or *L. casei*.
15. (original) A modified bacteria (other than *L. casei* or *Bacillus*) which has been modified to express a heterologous surface layer (S-layer) protein.
16. (original) A bacteria according to claim 15 which would not normally, or as a wild-type or in unmodified form does not, possess a surface layer.
17. (previously presented) A modified bacteria according to claim 15 which is a *Lactobacillus* cell and/or the S-layer has its own, original, cell wall anchor.

18. (previously presented) A bacteria according to claim 15 which is a *Lactobacillus* bacterial cell, such as *L. casei*, and/or the S-layer protein is from *Lactobacillus* bacteria, such as *L. acidophilus*.

19. (previously presented) An *L. casei* bacterial cell expressing a bacterial surface layer (S-layer) protein that is either not from *L. crispatus* or is not a collagen binding protein.

20. (original) An *L. casei* cell according to claim 19 wherein the S-layer protein is, or is derived, from *L. acidophilus*.

21. (original) A modified bacteria expressing only, or homogeneously, a heterologous or modified surface layer (S-layer) protein.

22. (original) A bacteria according to claim 21 having a genome which includes a polynucleotide encoding a heterologous S-layer protein, optionally integrated into the genome, and/or where the polynucleotide encoding the normal or wild-type S-layer protein has been silenced, replaced, switched off or otherwise rendered non-expressed.

23. (previously presented) A bacteria according to claim 22 wherein the heterologous or modified S-layer protein is the sole or only S-layer protein expressed by the bacterial cell and/or the cell does not express any wild-type S-layer protein.

24. (previously presented) A bacteria according to claim 22 wherein the S-layer protein is located on the surface of the cell wall and/or a multiplicity of S-layer proteins form an S-layer.

25. (previously presented) A vaccine comprising a bacteria according to claim 13, a modified bacteria (other than *L. casei* or *Bacillus*) which has been modified to express a heterologous surface layer (S-layer) protein, an *L. casei* bacterial cell expressing a

bacterial surface layer (S-layer) protein that is either not from *L. crispatus* or is not a collagen binding protein or a modified bacteria expressing only, or homogeneously, a heterologous or modified surface layer (S-layer) protein.

26. (original) A vaccine according to claim 25 which is an oral or nasal vaccine and/or additionally comprises an adjuvant.

27. (previously presented) A sheet or (optionally crystalline) monolayer or 2-dimensional array comprising a plurality of bacterial surface layer proteins, at least one of which is modified protein according to claim 1.

28. (original) A solid surface, liquid-air interface, lipid film, liposome or solution comprising a sheet, monolayer or array according to claim 27.

29. (original) A solid surface according to claim 28 to which is bound one or more (macro) molecules, such as an enzyme, antibody or other binding molecule, receptor, antigen or ligand.

30. (previously presented) A solid surface comprising a layer of S-proteins, at least a plurality of which are modified proteins according to claim 1, sandwiched between the surface and a layer of functional molecules.

31. (previously presented) A sensor, molecular sieve or ion trap comprising a sheet, layer or array according to claim 27 or a surface comprising a sheet, monolayer or array according to claim 27.

32. (previously presented) A sensor, molecular sieve or ion trap comprising a solid surface comprising a layer of S-proteins, at least a plurality of which are modified

proteins according to claim 1, sandwiched between the surface and a layer of functional molecules.